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A STUDY OF TETANUS TOXINS AND TOXOIDS PREPARED IN CASEIN MEDIA

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In recent years numerous investigations have been published in the literature concerning the cultivation of bacteria in semisynthetic and synthetic media. Among these the papers concerning the preparation of toxins and toxoids in semisynthetic media are of special interest. The most important among the latter is casein in form of an acid hydrolysate or a tryptic digest (Gladson and Fields, 1940; Finney 1943; Fizek, 1943; Müller and Miller, 1947, 1953, 1954). According to the opinion of these authors the use of semisynthetic media makes it possible systematically to obtain toxins of high activity; the toxoids prepared in such media show a high degree of antigenic and immunogenic properties and can easily be concentrated and purified.

Until recently meat media were used in the Soviet Union for the mass production of tetanus toxoid prepared by modifications of the methods of Ramon and Gluzman. These media are unsuitable for the mass production of tetanus toxin, not only because they are expensive, but mainly because it is impossible systematically to obtain from these media tetanus toxin of high quality. In order to lower the price of the distributed preparation and obtain a toxoid of higher quality we replaced the meat media by casein.

In preliminary investigations, trials were made of 20 different formulae for culture media the main constituent of which was casein hydrolysates. The best medium for the production of tetanus toxin was a medium prepared from casein hydrolysate with the addition of wheat husk and aqueous yeast extract.

In this medium 40 batches of tetanus toxin (about 2,000 l.) were prepared. The strength of the toxins obtained from the casein media was 2–3 times greater than the strength of toxins prepared in the media of Ramon and Gluzman. The antigenic properties of the casein toxoids were also considerably higher than those of the antigens prepared in meat media. Whereas our production toxins prepared in Ramon medium contained an average of 750,000 Dlm and 75 F.U. per ml and the toxins made in Gluzman medium 1,000,000 Dlm and 100 F.U., the toxins made in casein media contained an average of 2,000,000 Dlm and 200 F.U.

A shorter time is needed for the inactivation of toxins prepared in media not containing meat than of toxins prepared in Ramon or Gluzman medium. Whereas toxin in Glazman medium could be inactivated in 20–25 days and in Ramon medium in 18–21 days, the inactivation of toxins prepared in casein media took place in 15–16 days.

In an investigation of the antigenic properties of toxoids prepared in media not containing meat it was found that the fixing capacity of the toxins corresponded to the fixing capacity of the toxoids.

The characteristic properties of 16 batches of tetanus toxins are given in Table 1, including strength and fixing capacity as well as the antigenic properties of the toxoids prepared from these batches of tetanus toxins.

In experiments on guinea-pigs the degree of immunity developing as a result of the infection of toxoids prepared in casein media was investigated. Each batch was tested on 3, 4, or 5 guinea-pigs which were each injected with 5 ml of toxoid. Thirty days later the animals were each given 100, 200 or 500 Dlm of tetanus toxin.

The experiment showed (Table 2) that all the animals immunized with tetanus toxoid of batches nos. 35, 40, 52, 54, 55, 58, and 59 survived the injection of 100 Dlm

TABLE 1. CHARACTERISTIC PROPERTIES OF TETANUS TOXINS AND TOXOIDS PREPARED IN CASEIN MEDIA

No. of batch	Dlm	F.U. of the toxin	F.U. of toxoid
35	3,000,000	> 250 < 300	> 250 < 300
40		300	300
52	3,000,000	200	200
58	800,000	100	100
64	2,500,000	300	300
74	3,000,000	300	300
77	2,500,000	> 200 < 300	> 200 < 300
81	2,500,000	> 200 < 300	> 200 < 300
84	5,000,000	> 200 < 300	275
86	2,000,000	> 200 < 300	> 200 < 300
89	5,000,000	400	400
91	2,000,000	100	100
97	1,000,000	125	125
109	1,000,000	100	100
113	2,000,000	200	200
137	2,000,000	200	200

of toxin without any signs of tetanus. The guinea-pigs which had been given toxoid of batches no. 64, 74, 77, 81, and 84 were injected a month later with 100 and 200 Dlm of toxin. Six days after the injection of the challenge dose one guinea-pig showed signs of first degree tetanus. In addition 4 guinea-pigs immunized with toxin of batch no. 84 were injected with 500 Dlm of toxin. Three showed no signs of tetanus, but one developed second degree tetanus.

TABLE 2. DEGREE OF IMMUNITY IN GUINEA-PIGS AFTER A SINGLE INJECTION OF TETANUS TOXOID PREPARED IN CASEIN MEDIA

Time of investigation	No. of batch	Dose of toxoid (ml)	Total number of guinea-pigs	100 Dlm			200 Dlm			300 Dlm		
				Result			Result			Result		
				Number of guinea-pigs	No signs	Signs of tetanus	Number of guinea-pigs	No signs	Signs of tetanus	Number of guinea-pigs	No signs	Signs of tetanus
After preparation	35	5	4	4	4	—	—	—	—	—	—	—
	40	5	4	4	4	—	—	—	—	—	—	—
	52	5	4	4	4	—	—	—	—	—	—	—
	54	5	4	4	4	—	—	—	—	—	—	—
	55	5	4	5	5	—	—	—	—	—	—	—
	58	5	3	3	3	—	—	—	—	—	—	—
	59	5	3	3	3	—	—	—	—	—	—	—
	64	5	4	3	2	Tet 1	1	1	—	—	—	—
	74	5	4	3	3	—	2	2	—	—	—	—
	77	5	3	2	2	—	1	1	—	—	—	—
	81	5	3	3	3	—	2	2	—	—	—	—
	84	5	8	2	2	—	2	2	—	4	3	Tet 2
After 1 year storage	64	5	3	1	1	—	2	2	—	—	—	—
	74	5	3	2	2	—	1	1	—	—	—	—
	81	5	3	1	1	—	2	2	—	—	—	—

The antigen content and antigenicity of the three batches of tetanus toxoid nos. 64, 74, and 81 obtained in casein media were tested 1 year after they had been prepared. The antigenic strength of these batches of toxoids on the second determination was as follows: batch no. 64: 300 F.U., batch no. 74: 300 F.U. and batch no. 81: >200-<300 F.U. per ml. The redetermination showed that the antigen contents of these batches of tetanus toxoid had not changed during 1 year of storage. The degree of immunity provided by each of these three batches of toxoid was investigated in 3 guinea-pigs, which received 5 ml of toxoid subcutaneously in the foot. After 30 days 100 or 200 DLm of tetanus toxin was injected into the animals, and was completely neutralized by the serum of the immunized animals (Table 2). The experiment showed that 1 year after preparation the antigenicity of the toxoid had also remained at the previous level.

In addition, four batches of tetanus toxoid (nos. 64, 90, 97, and 98) were used for the immunization of guinea-pigs, the blood of which was later examined for its antitoxin content. The animals received two injections each of crude toxoid in a volume of 1 ml with an interval of 21 days. Thirty days after the second injection the blood of the guinea-pigs was examined.

As a result of the immunization of 5 guinea-pigs with toxoid of batch no. 64 containing 300 F.U. per ml the serum of 4 animals contained >1/10<1 and of one animal >1<5 A.U. per ml. Among 5 animals which had been immunized with tetanus toxoid of batch no. 90 containing 175 F.U. per ml the serum of 4 contained >1/10 <1 and of one >1 <3 A.U. After immunization with tetanus toxoid of batch no. 98 containing 175 F.U. per ml less than 1/10 A.U. was found in one guinea-pig, >1/10 <1 in 2 guinea-pigs and >1 <3 A.U. in 2 guinea-pigs (Table 3).

TABLE 3. DEVELOPMENT OF ANTITOXIN IN THE SERUM OF GUINEA-PIGS AFTER IMMUNIZATION WITH TWO INJECTIONS OF TETANUS TOXOID PREPARED IN CASEIN MEDIA

No. of batch of toxoid	Amount of toxoid (ml) used for one injection	Number of F.U. in 1 ml of antigen	Number of guinea-pigs	Number of A.U. in 1 ml. of the animal's serum
64	1	300	4	>1/10<1
			1	>1<5
90	1	175	4	>1<3
			1	>1/10<1
97	1	125	4	>1/10<1
			1	>1<3
98	1	175	2	>1/10<1
			1	1/10
			2	>1<3

We further investigated the immunizing properties of tetanus toxoids prepared in casein media and adsorbed on aluminium hydroxide.

The adsorptive capacity of the aluminium hydroxide was first determined. For this purpose we added to 7 batches of tetanus toxoid containing 100–300 F.U. per ml aluminium hydroxide in the amount of 2·5 mg of Al_2O_3 per ml of antigen. The completeness of adsorption was assessed from the amount of residual toxoid in the supernatant. Table 4 shows that in the 6 series of tetanus toxoid examined, not less than 5 and not more than 50 F.U. per ml remained in the supernatant, depending on the antigen content of the toxoids.

Having convinced ourselves of the fact that the tetanus toxoids prepared in casein medium are well adsorbed we carried out an immunization experiment on guinea-pigs with various batches of tetanus toxoid adsorbed on aluminium hydroxide. Crude toxoid of batch no. 64 contained 300 F.U. per ml; after adsorption with aluminium hydroxide only 25 F.U. of toxoid remained in the supernatant fluid. Toxoid of batch no. 90 contained 175 F.U. per ml, but the supernatant fluid after adding aluminium hydroxide

Table 4. Adsorption of tetanus toxoid prepared in casein media on aluminium hydroxide

No. of batch of toxoid	No. of F.U. in 1 ml of toxoid	Amount of Al_2O_3 per ml of toxoid (mg)	No. of F.U. per ml of supernatant
64	300	2·5	$>10<50$
81	250	2·5	$>10<50$
84	275	2·5	$>10<50$
90	150	2·5	$>5<10$
97	125	2·5	$>25<50$
98	175	2·5	$>25<50$

contained only 5 F.U. The fixation capacity of the toxoid of batch no. 97 was 125 F.U., and in the supernatant fluid there were 25 F.U. In 1 ml of toxoid of no. 98 there were 175 F.U., but after adsorption with aluminium hydroxide the supernatant fluid contained 25 F.U.

Two groups of animals were immunized with each antigen, some with crude antigen and others with adsorbed crude antigen. The guinea-pigs were immunized with two

Table 5. Immunizing properties of crude and adsorbed crude tetanus toxoids prepared in casein media

Batch no. of antigen	No. of F.U. per ml of crude toxoid	No. of F.U. per ml of completely adsorbed toxoid	No. of F.U. per ml of supernatant	No. of guinea-pigs	No. of A.U. per ml of serum
64	300	—	—	5	>1/10<1
		275	25	5	13·5
90	175	—	—	5	>1/10<1
		170	5	2	15
97	125	—	—	4	>1/10<1
		100	25	5	10
98	175	—	—	5	>1/10<1
		150	25	5	5·5

injections of 1 ml of the preparation with an interval of 21 days. The blood for the determination of the antitoxin was taken 30 days after the second injection. The experiment showed the superiority of the adsorbed crude antigen over the unadsorbed crude antigen. After immunization with unadsorbed crude toxoid the antitoxin content of the serum ranged from >1/10 to <1 A.U., whereas in animals immunized with adsorbed crude toxoid the serum contained a much higher amount of antitoxin (Table 5). After immunization with adsorbed crude toxoid of batch no. 64 the average titre of the serum was 13 A.U. per ml (5 guinea-pigs), and after immunization with toxoid of batch no. 90 the serum contained 15 A.U. (2 guinea-pigs), with toxoid of batch no. 97, 10 A.U. (5 guinea-pigs), and with toxoid of batch no. 98, 5·5 A.U. (5 guinea-pigs).

On the basis of these results we are able to recommend for the mass production of tetanus toxoid a medium the main constituent of which is casein hydrolysate. In this medium, tetanus toxoids with high antigen content and antigenicity can be prepared.

CONCLUSIONS

(1) Tetanus toxins prepared in casein media have a toxicity of 2 or 3 times greater strength than toxins prepared in Ramon and Gluzman media.

(2) Tetanus toxoids prepared from casein media have an antigen content 2 or 3 times greater than toxoids prepared from meat media.

(3) The inactivation of tetanus toxins prepared in casein media can be achieved in a shorter time than the inactivation of toxins prepared in meat media.

(4) The fixation capacity of tetanus toxins prepared in casein media ran parallel to the fixation capacity of the toxoids expressed in F.U. (fixation units).

(5) The immunizing properties of crude tetanus toxoids prepared in casein media satisfied the standards laid down in the instructions for the preparation of tetanus toxoids in meat media.

(6) Re-investigation carried out 1 year after preparation showed that the antigen content and antigenicity of toxoids prepared in casein media was fully retained.

(7) Crude tetanus toxoids prepared in casein media are well adsorbed by aluminium hydroxide.

(8) The immunizing properties of tetanus toxoids adsorbed on aluminium hydroxide are higher than the immunizing properties of crude toxoids.

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THE COURSE OF EXPERIMENTAL LEPTOSPIROSIS IN IRRADIATED ANIMALS

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IT IS generally known at the present time that after irradiation with ionizing radiation the mechanisms of immunity are damaged. The phagocytic activity of the reticulo-endothelial cells is suppressed (Pestova, Alekseeva, Wilkinson), the bactericidal properties of fluids and tissues diminish (Klemparskaia, Markus), antibody formation is depressed (Hekton, Taliaferro, Kosov), the permeability of vessels and tissues increases (Gudz, Kiselev, Gorizontov), and the functions of the vegetative and central nervous system undergo a change (Abramenko, Bykin, Lebedinskii).

It is natural that irradiated animals become more susceptible to infection by infectious agents, and that the course of the infection is more severe. This is substantiated by many data. Saxton found a more rapid and severe course of tuberculosis in irradiated rabbits. Korper discovered an increased susceptibility to pneumococcal and streptococcal infections in irradiated white mice. Similar data are available concerning trypanosome infection (Neiman), mouse typhoid (Gouin), rickettsiosis (Zinsser), and virus infections (Beutler, Smorodintsev, etc.). We found no data in the literature concerning spirochaetosis in irradiated animals.